Synthesis and Characterization of ZnO nanoparticles of various sizes and Applications in Biological systems

Thesis submitted in partial fulfillment

of the requirements for the degree of

Bachelor of Technology

in

Biotechnology

by

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Certificate

This is to certify that the thesis entitled, "Synthesis and characterisation of ZnO nanoparticles with various size and their application in biological system" submitted by Mr. Omkar Behera (107BT017) in fulfillments for the requirements for the award of Bachelor of Technology degree in Biotechnology and Medical Engineering at National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

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ACKNOWLEDGEMENT

With deep regards and profound respect, I take this opportunity to express my deep sense of gratitude and indebtedness to Prof. Subhankar Paul, Head of the Department, Department of Biotechnology and Medical Engineering, N. I. T. Rourkela, for introducing the research topic and for inspiring guidance, constructive criticism and valuable suggestion throughout this research work. It would have not been possible for me to bring out this project report without his help and constant encouragement. I wish that he will keep in touch with me in future and will continue to give his valuable advice. I am also grateful to all the faculties of Department of Biotechnology and Medical Engineering, whose vast knowledge in the field of Biotechnology and Medicals has enlightened me in different areas of this research work. I am also thankful to Mr Vibhanshu Maheswari and other research scholars in Department of Biotechnology and Medical Engineering, Abhishek Paul, Suraj Dash, Akash Satapathy,Animesh chottray, Biswajit Maharathi, Deepika Rani Mittal for providing the joyful environments in the lab and helping me. It was a nice and memorable experience with all the things of my department. I wish to give them my heartfelt thanks for their constant help and support. I would also like to thank my mother for constantly encouraging and supporting me to give my 100 % and work hard till I achieve my goal. Above all, I thank the Almighty GOD for giving me all these people to help and encourage me, and for the skills and opportunity to complete this report

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Abstract

In the present research work, synthesis of zinc nanoparticles and its characterization was done. In this study, zinc nanoparticles were rapidly synthesized from Zn(NO3)2 solution using soluble starch and formation of nanoparticles observed with in precipitation method. The white synthesized zinc Nanoparticles were characterized by different spectroscopic and analytical techniques such as XRD and SEM. X-ray diffraction and SEM analysis studies confirmed the formation of well-dispersed zinc nanoparticles with average particle size to be in the range of 35 to 41 nm as well as revealed their hexagonal structure. These biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens. Therefore different sized synthesized zinc nanoparticles were used to study the antimicrobial activity against Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, and Staphylococcus aureus by using well-diffusion and disc diffusion methods and the bacterial growth was monitored by measurement of optical density (OD) of solution and estimation of colony forming units (CFU) on solid growth medium. The result showed that the antimicrobial activity increased with the decrease of the size of the nanoparticles.

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Introduction

1 Introduction

Zinc oxide, ZnO is an inorganic compound also known as zincite and occurs rarely in nature, generally in a crystalline form. It is usually orange or red in color due to presence of manganese impurity. It usually appears as a white crystalline powder, which is nearly insoluble in water. Most of ZnO which is used commercially is produced synthetically. ZnO is actually a wide-bandgap semiconductor of the II-VI semiconductor group. The doping of the semiconductor is n-type which is due to oxygen vacancies. This has several favorable properties like high electron mobility, good transparency, wide bandgap for seni-conductivity, high room-temperature luminescence, etc. These properties are used in applications for electrodes in liquid crystal displays as well as in energy-saving and heat-protecting windows[1], electronic applications of ZnO as thin-film transistors and light-emitting diodes and also in ceramics plastic Zinc oxide (ZnO) has a stable wurtzite structure[1] with lattice spacing a = 0.325 nanomtres and c = 0.521 nanometres. Because of its unique properties and versatile Applications, it is used in transparent electronics, ultraviolet (UV) light emitters, piezoelectric devices and chemical sensors. These remarkable physical properties form the basis for motivation of device miniaturization, large effort has been focused on the synthesis, characterization and device applications of ZnO nanomaterials.

Properties Of ZnO

2 Physical Properties

ZnO posseses the following physical properties[2]: • Molecular Weight : 81.37

• Color : Pure microcrystalline zinc oxide is white. Single crystal zinc oxide is colorless. Zinc oxide turns lemon yellow on heating and reverts to white on cooling.

• Relative Density : 5.607

• Melting Point : Zinc oxide sublimes at atmospheric pressure at temperatures over 1200^{o}C. Under high pressure a melting point of 1975^{o}C has been estimated.

• Vapor Pressure(1500^{o}C): 12mm.

• Refractive Index : w = 2.004, e = 2.020

• Heat of Sublimation between 1350^{o}C and 1500^{o}C :- 129 Kcal/mole (vapor not disassociated) and 193 Kcal/mole (vapor associated).

• Heat Capacity:Cp = 9.62 cal/deg/mole at 25^{o}C

• Coeffcient of Thermal Expansion = 4 x 10(-6)/^{o}C

2.1 Crystal Structure

Zinc oxide has the wurtzite hexagonal crystal structure. Commercial zinc oxides show this crystal structure only under electron microscopic examination. The precise shape of the crystal depends on the method of formation. In regular zinc oxide these vary between acicular needles and plate shaped crystals. Zinc oxide can be induced to form a very large variety[2] of crystalline shapes using specialized deposition methods, which is currently a very active area of research.

Zinc oxide usually crystallizes in three different forms: hexagonal wurtzite, cubic zincblende and cubic rocksalt. The latter is the most rarely found .The wurtzite structure is most stable at ambient conditions and is hence most common.

2.2 Mechanical Properties

ZnO is a relatively very soft material with approximate hardness just 4.5. Its elastic constants are relatively smaller than those of other III-V semiconductors, e.g. GaN. The high heat capacity and high heat conductivity, low values of thermal expansion and high melting points are

some of the characteristics of ZnO. ZnO has been proposed to be a more promising UV emitting phosphor than GaN because of its larger exciton binding energy (60 meV). Among the semiconductors bonded tetrahedrally, its found that ZnO has the highest piezoelectric tensor. This makes it an important material for many piezoelectric applications, which require a high degree of electromechanical coupling among them.

2.3 Piezoelectric effect and Polar Surfaces

Piezoelectricity of ZnO has been extensively studied for various applications in force sensing, acoustic wave resonator, acousto-optic modulator, etc. The origin of the piezoelectricity lies due to its crystal structure, in which the oxygen atoms and zinc atoms are tetrahedrally bonded. In such a structure whch is non centrosymmetric, the center of positive charge and negative charge can be displaced due to external pressure induced lattice distortion. This displacement results in local dipole moments, thus a macroscopic dipole moment appears over the entire crystal. In fact, among the tetrahedrally bonded semiconductors, ZnO has [3] the highest tensor which provides a large electro-mechanical coupling. Another result of the non-centrosymmetric ZnO crystal structure is its spontaneous polarization and polar face dominated nanostructures. The crystal structure of ZnO can be visualized in a way that oxygen atoms and zinc atoms are tetrahedrally bonded. These tetrahedrons stack along a particular direction. Due to the effect of spontaneous polarization, the position of positive charge is displaced from that of negative charge in that fixed particular direction. The net result of this spontaneous polarization is a charged ZnO surface. In order to achieve minimized energy, the charged surface results in unique nano-ring and nano-coil structure.

2.4 Electrical Properties

The fundamental study of the electrical properties of ZnO nanostructures is crucial for developing their future applications in nanoelectronics. ZnO has a quite large band gap of 3.3 eV at room temperature. The advantages of a large band gap include higher values of breakdown voltages, sustaining large electric fields, high-temperature and high-power operations. ZnO has n-type character, in the absence of doping. Non-stoichiometry is usually the origin of n-type character.

Due to defects such as oxygen vacancies and zinc interstitials, ZnO nanowires are reportedly show n-type semiconductor behavior. The main impediment of ZnO for wide-ranging applications in electronics and photonics rests with the difficulty of p-type doping. Successful p-type doping for ZnO nanostructures will greatly enhance their future applications in nanoscale electronics and optoelectronics. P-type and n-type ZnO nanowires can serve as p-n junction diodes and light emitting diodes (LED). [4]

2.5 Optical Properties

Zinc oxide[2] is generally transparent to visible light but strongly absorbs ultra violet light below 3655 A. The absorption is typically stronger than other white pigments. In the region of visible wavelengths, regular zinc oxide appears white, but, rutile and anatase titanium dioxide have a higher reactive index and thus has a superior opacity. The band gap energy (between valence and conducting bands) is 3.2 ev, this corresponds to the energy of 3655 A photons. Under ultra violet light zinc oxide is photoconductive. The combination of optical and semiconductor properties make doped zinc oxide a contendor for new generations of devices. Solar cells require a transparent conductive coating, indium tin oxide and zinc oxide(doped) are the best materials.

Intrinsic optical properties of ZnO nanostructures are being intensively studied for implementing photonic devices. Photoluminescence (PL) spectra of ZnO nanostructures have been extensively reported. Excitonic emissions have been observed from the photoluminescence spectra of ZnO nanorods. It is shown that quantum size confinement can significantly enhance the exciton binding energy. Strong emission peak at 380 nm due to band-to-band transition and green-yellow emission band related to oxygen vacancy are observed. PL spectra show that ZnO nanowire is a promising material for UV emission, while its UV lasing property is of more significance and interest. Due to its near-cylindrical geometry and large refractive index (˜2.0), ZnO nanowire/nanorod is a natural candidate for optical waveguide. The additional advantages of ZnO nanowire lasers are that the excitonic recombination lowers the threshold of lasing, and quantum confinement yields a substantial density of states at the band edges and enhances radiative efficiency. Optical waveguiding using dielectric nanowire also achieved considerable progress. Recently, ZnO nanowires were reported as sub-wavelength optical waveguide. Optically pumped

light emission was guided by ZnO nanowire and coupled into SnO2 nanoribbon. These findings show that ZnO nanostructures can be potential building blocks for integrated optoelectronic circuits.[5]

3 Chemical Properties

ZnO has the following chemical properties[2]:

• ZnO occurs as the mineral zincite or as white powder known as zinc white. It is usually orange or red in color due to manganese impurity.

• Crystalline zinc oxide is thermochromic, which changes from white to yellow colour when heated and reverting to white colour on cooling. This change in colour is caused by a very small loss of oxygen at high temperatures.

• Zinc oxide is amphoteric, that is it reacts with both acids and alkalis. With acid it reacts to form familiar compound such as zinc sulfate. With alkali it forms zincates.

$ZnO + 2\ HCl \rightarrow ZnCl_2 + H_2O$

$ZnO + 2\ NaOH + H_2O \rightarrow Na_2(Zn(OH)_4)$

• ZnO decomposes to form zinc vapor and oxygen at about 1975 $\mathring{A}$ ^oC, indicating its considerable stability. Heating with carbon converts ZnO into Zn, which is more volatile.

$ZnO + C \rightarrow Zn + CO$

• The following reaction is extremely important in zinc pyrometallurgy-

$ZnO + CO \rightarrow Zn + CO_2$

• Commercial zinc oxide shows a measurable but low level of water solubility, 0.005 g/litre.

• Zinc oxide exposed to air absorbs both water vapour and carbon dioxide. This results [6] in the formation of basic zinc carbonate.

Literature Review

4 Nanotechnology and Nanomaterials

Nanotechnology[4] is defined as the study of manipulating matter on the atomic and molecular scale. In General, nanotechnology deals with structures whose sizes vary between 1 to 100 nm in one dimension at least, and involves developing materials having at least one dimension within that size range. It covers various areas ranging from conventional device physics to completely new approaches based on molecular self-assembly, from developing materials having dimensions of the nanoscale to finding out whether we can control matter on the atomic scale. It is able to create many new materials with a vast range of applications, such as in medicine, biomaterials, electronics, and production of energy. However, nanotechnology raises many concerns about toxicity and impact of nanomaterials on environment, and their effects on global economics.

Nanoparticles are particles that have one dimension that is 100 nanometers or less in size.The properties of many conventional materials change when formed from nanoparticles. This is typically because nanoparticles have a greater surface area per weight than larger particles; this causes them to be more reactive to certain other molecules.Nanoparticles are used, or being evaluated for use, in many fields.

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed. Thus, the properties of materials generally change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material starts to become significant. For bulk materials larger than one micrometer (or micron), the percentage of [7] atoms at the surface is insignificant in comparision to the number of atoms in the bulk of the material. The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material. Nanoparticles often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. Other size-dependent property changes int he nanoparticle include quantum confinement in semiconductor particles, surface plasmon

resonance in some metal particles and superparamagnetism in magnetic materials.

4.1 Properties of Nanoparticles

• A bulk material should have constant physical properties regardless of its size, but at the nanoscale size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant.

• For bulk materials larger than one micrometer (or micron), the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material. The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material.

• Nanoparticles of usually yellow gold and gray silicon are red in color; gold nanoparticles melt at much lower temperatures (nearly 300^o C for 2.5 nm size) than the gold slabs (1064 ^{o}C); and absorption of solar radiation in photovoltaic cells is much higher in materials composed of nanoparticles than it is in thin films of continuous sheets of material , the smaller the particles, the greater the solar absorption.

• Suspensions of nanoparticles are possible since the interaction of the particle surface with the solvent is strong enough to overcome density differences, which otherwise usually [8] result in a material either sinking or floating in a liquid.

• Nanoparticles also often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. For example gold nanoparticles appear deep red to black in solution.

• The high surface area to volume ratio of nanoparticles provides a tremendous driving force

for diffusion, especially at elevated temperatures.

• Moreover, nanoparticles have been found to impart some extra properties to various day to day products. For example the presence of titanium dioxide nanoparticles imparts what we call the self-cleaning effect, and the size being nanorange, the particles can not be observed. Zinc oxide particles have been found to have superior UV blocking properties compared to its bulk substitute. This is one of the reasons why it is often used in the preparation of sunscreen lotions, and is completely photostable.

4.2 Advantages Of Nanoparticles

The advantages of using nanoparticles include the following:

• Particle size and surface characteristics of nanoparticles can be easily changed to achieve both passive and active drug targeting after parenteral administration.

• They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.

• Controlled release and particle degradation characteristics can be readily odulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for [9] preserving the drug activity.

• Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.

• The system can be used for various routes of administration including oral, nasal, parenteral,intraocular etc.

4.3 Limitations of Nanoparticles

In spite of these advantages, nanoparticles do have limitations.

• For example, their small size and large surface area can lead to particleparticle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms.

• In addition, small particles size and large surface area readily result in limited drug loading and burst release.

These practical problems have to be overcome before nanoparticles can be made commercially available. The present review details the latest development of nanoparticulate drug delivery systems, surface modification issues, drug loading strategies, release control and potential applications of nanoparticles.

4.4 Synthesis of Nanoparticles

There are several methods for creating nanoparticles, including both attrition and pyrolysis.

In attrition, macro or micro scale particles are ground in a ball mill, a planetary ball mill, or other size reducing mechanism. The resulting particles are air classified to recover nanoparticles.

In pyrolysis, a vaporous precursor (liquid or gas) is forced through an orifice [10] at high pressure and burned. The resulting solid (a version of soot) is air classified to recover oxide particles from by-product gases.

Pyrolysis often results in aggregates and agglomerates rather than single primary particles. A thermal plasma can also deliver the energy necessary to cause evaporation of small micrometer size particles. Inert-gas condensation is frequently used to make nanoparticles from metals with low melting points.

4.4.1 Fundamental Techniques For Synthesizing Nanomaterials

• Form in place:This includes vacuum coating, lithography and spray coating.

• Mechanical :This method reduces the size of particles by ball milling or by planetary grinding.

• Gas phase synthesis: These include chemical vapor synthesis,plasma vaporization and laser ablation.

• Wet chemistry: These techniques are generally applicable for characterization by light scattering techniques.

These are basically bottom-up approaches, that is, they begin with ions or molecules and gradually build them up into larger structures. These nanoparticle synthesizing techniques come under the colloid chemistry, and also involve classical sol-gel processes, or other aggregation processes. These wet chemistry techniques synthesize the best quality nanoparticles. This is because of the following reasons:

• This method produces nanoparticles which are in the form of a dispersion, hence high interparticle forces can be designed in order to prevent agglomeration.

• No formation of aggregates.[11]

• The nanoparticles are monodisperse, that is, all are of the same size with small tolerances.

• The morphology and chemical composition can be minutely controlled. This is important for research purposes where the material quality must be very high to derive meaningful results.

4.4.2 Selection of matrix materials

Nanoparticles [8] can be prepared from a wide variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is generally dependent on many factors including:

(1) size of nanoparticles

(2) inherent properties , e.g., aqueous solubility and stability

(3) surface characteristics such as charge,permeability etc

(4)Drug release profile that is desired

(5) Antigenicity of the final product

Nanoparticles [8] have been prepared generally by three methods:

(a) dispersion of preformed polymers

(b) polymerization of monomers

(c) ionic gelation or coacervation of hydrophilic polymers.

4.5 Methods Of Synthesis

4.5.1 Solvent evaporation method

In this process, the polymer is dissolved in an organic solvent which is also used to dissolve the water repellant drug. The mixture containing the drug solution and polymer is then emulsified in an solution containing a emulsification agent to form an emulsion of oil in water. After the formation of emulsion, the organic solvent is then evaporated by continuously stirring the solution or by reducing the pressure. Particle size is influenced by homogenizer speed, the type and concentrations of stabilizer and polymer concentration.

4.5.2 Spontaneous emulsification or solvent diffusion method

This is a modified version of the earlier method. Usually the water miscible solvent, together with a small quantity of the organic solvent is serves as the oil phase. Due to the diffusion of solvents, an interfacial turbulence is generated between the 2 phases which in turn leads to the formation of small sized particles. As the quantity of solvent increases, a further decrease in the particle size can be achieved. [12]

4.5.3 Polymerization Method

In this method, monomers are polymerized to form nanomaterials in an aqueous solution . Drug is incorporated by adsorption onto the nanoparticles or by dissolving in the polymerization medium after polymerization has been completed. The suspension is then purified to remove stabilizers and surfactants which were used for polymerization by ultracentrifugation and then re-suspending the particles in a mdium free of isotonic surfactants. This technique is suitable for making poly (alkylcyanoacrylate) or polybutylcyanoacrylate nanoparticles. The particle size depends on the conc. of the stabilizers and surfactants used. [12,13]

4.5.4 Coacervation Or Ionic Gelation Method

Much research has focussed on the preparation of nanoparticles from biodegradable hydrophilic polymers such as gelatin, chitosan and sodium alginate. The method involves a mixture of two aqueous phases, of which is the polymer Chitosan and the other Is the polyanion sodium

tripolyphosphate. In this technique, amino group of chitosan combines with tripolyphosphate to form coacervates with a size in the range of nanometers. These are formed due to electrostatic interaction between the two phases, while ionic gelation involves transition from liquid phase to the gel phase due to ionic interaction which takes place at room temperature.[12,13]

4.5.5 Synthesis of nanoparticles using supercritical fluid technology

Conventional methods such as solvent diffusion, solvent extraction-evaporation and organic phase separation methods require organic solvents which are harmful to the environment. Therefore, the supercritical fluid technology is generally used as an alternative for preparing biodegradable micro and nanoparticles because supercritical fluids are considered to be environmentally safe. A supercritical fluid can be generally defined as a solvent at a value higher than its critical temperature, at which the it remains in a single phase irrespective of pressure. Supercritical CO_2 is the most widely used super critical fluid because of its relatively mild critical conditions (T_c = 31.1 ^{o}C, P_c = 73.8 bars), non-flammability, nontoxicity and lesser cost.

4.5.6 RESS And SAS

The most common processing techniques involving supercritical fluids are rapid expansion of critical solution (RESS) and supercritical anti-solvent (SAS). This method employs a liquid solvent like methanol, which is miscible with the supercritical fluid to dissolve the solute to be nanomaterialized. This is because the solute is not soluble in the supercritical fluid. The extract of the liquid solvent by supercritical fluid results in instantaneous precipitation of the solute, thus leading to the formation of nanoparticles.[14]

RESS varies from the SAS process in the fact that its solute is first dissolved in a supercritical fluid and then the solution is quickly expanded through a nozzle into a region of lower pressure. Thus the dissolving power of supercritical fluids decreases a lot and eventually the solute precipitates.

This method is basically clear because the precipitate is solvent free. RESS and its modified processes have been used since a long time for the production of polymeric nanoparticles. Supercritical fluid technology, though environment-friendly and also suitable for mass production,

however requires specially designed equipments and is also more expensive.

5 Characterization of Nanoparticles

When the dimensions of materials are reduced to nanoscale, they demonstrate unique properties which are far different from those of their bulk counterparts. For example, their electronic and optical properties change, their chemical properties can be increased or decreased and mechanical/structural stabilities are changed dramatically. These features make nanoparticles attractive for unique sensing applications, and also at the same time cause complications in their characterization processes. Therefore, the challenge lies in finding the right characterization techniques that have the optimum capabilities for studying the characteristics of nanomaterials generated by these techniques.

A large number of techniques can be employed for nanoparticle characterization.Two of the techniques are presented here. In addition, applicability of these techniques for investigating different types of nanomaterials and their relevance to sensor technology have also been described. Nanoparticle characterization is of utmost significance to establish understanding and control of nanoparticle synthesis and applications. Characterization is done by using a variety of different techniques, mainly drawn from materials science. Common techniques are electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), x-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), matrix-assisted laser desorption/ionization time-of- flight mass spectrometry (MALDI-TOF), ultraviolet-visible spectroscopy, dual polarisation[15].

Synthesis of ZnO Nanoparticles

6 Protocols

6.1 Protocol 1

In order to synthesize the ZnO nanoparticles, stock solutions of zinc acetate $Zn(CH3COO)_2.2H_2O$ (0.1 M) was prepared in 50ml methanol under stirring. To this stock solution 25ml of NaOH (varying from 0.2 M to 0.5 M) solution prepared in methanol was added under continuous stirring in order to get the pH value of reactants between 8 and 11. These solutions was transferred into teflon-lined sealed stainless steel autoclaves and maintained at various temperatures in the range of 100 - 200 degree C for 6 and 12 hours under autogenous pressure. It was then allowed to cool naturally to room temperature. After the reaction was complete, the resulting white solid products were washed with methanol, filtered and then dried in air in a laboratory oven at 60 degree C.

6.2 Protocol 2

The zinc oxide nanoparticles were synthesized by wet chemical method using sodium hydroxide zinc nitrate as precursors and starch as stabilizing agent. • Different concentrations of starch were dissolved in 1000 ml of distilled water. Zinc nitrate, 29.748 g was added in the above solution.

• Then the solution was continuously stirred using magnetic stirrer to dissolve the zinc nitrate completely.

• After dissolution of zinc nitrate, 0.2 M of NaOH solution (25 ml) was added with constant stirring, drop by drop slowly touching the walls of the vessel.

• This reaction was allowed to continue for 2 Hours after complete addition of NaOH. After the reaction completed, the solution was then allowed to settle and the supernatant was then carefully discarded.

• The remaining solution was then centrifuged at 10, 000 X g for few minutes and the supernatant was again discarded.

• The nanoparticles obtained were then washed 3 times by distilled water to remove the byproducts and the excessive starch bound with the nanoparticles. Then the nanoparticles were dried at

approximately 80 degree C for overnight. While drying, zinc hydroxide was completely converted into zinc oxide.

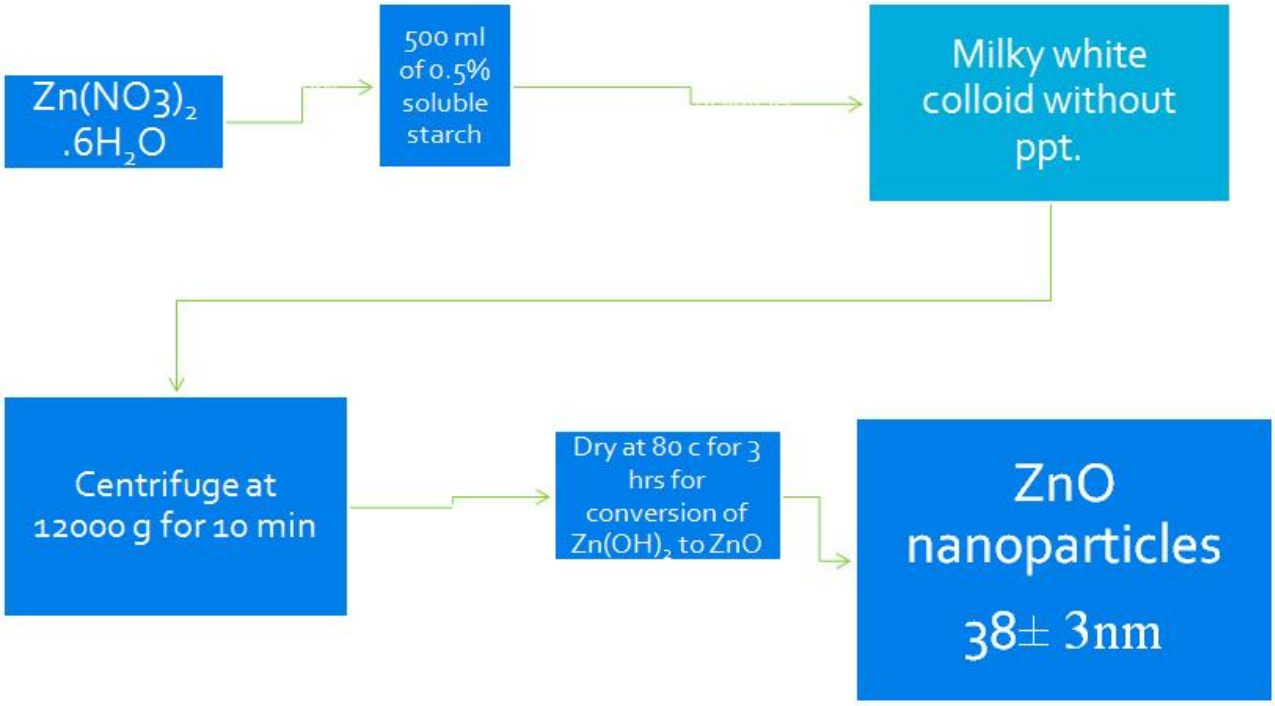

Figure 1: Schematic Diagram of ZnO synthesis

Results

7 Characterization of ZnO nanoparticles

7.1 X-ray Diffraction method

X-ray diffraction [1] is a non-destructive and analytical method for identification and quantitative analysis of various crystalline forms of ZnO, also known as phases of the compound present in the samples. Diffraction occurs when the waves collide with a regular structure in which the repeating distance is approximately same as the wavelength of the wave. It happens that X-rays have wavelengths n the order of a few angstroms. This means that the X-rays can be easily diffracted from materials which, are crystalline and have repeating and regular atomic structures. When the required parameters met, the X-rays that get scattered from a crystalline solid can interfere constructively, thus producing a diffracted beam of light. In 1912, W. L. Bragg derived a relationship among several factors:

(a) the interatomic spacing which Is known as d-spacing and is measured in angstroms.

(b) The angle of diffraction which is known as the theta angle and is measured in degrees.

(c) The wavelength of the incident X-rays, denoted by the lambda and, in this case, is equal to 1.54 angstroms.

$\mathbf{n\lambda = 2d \sin\Theta}$ λ- wavelength of X-ray

d-interplaner spacing,

Θ-diffraction angle

n-0,1,2,3,etc.

Here XRD was done by the X-ray diffraction of the samplesusing the diffractometer. X-Ray diffraction patterns were recorded from 20 degrees to 90 degrees with a PANalytical system diffractometer (Model: DY-1656) using CuK using an accelerating voltage of 40 KV. Data was collected at a rate of 1 degree/min. The $K_{doublets}$ were then resolved. The size of the crystallites was found out using the scherrers formula

$\mathbf{P = 0.9\lambda \ / \ \beta \cos \theta}$ P - crystallite size

λ - wavelength(1.54 Armstrong)

β - Full maxima half width

θ - Diffraction angle

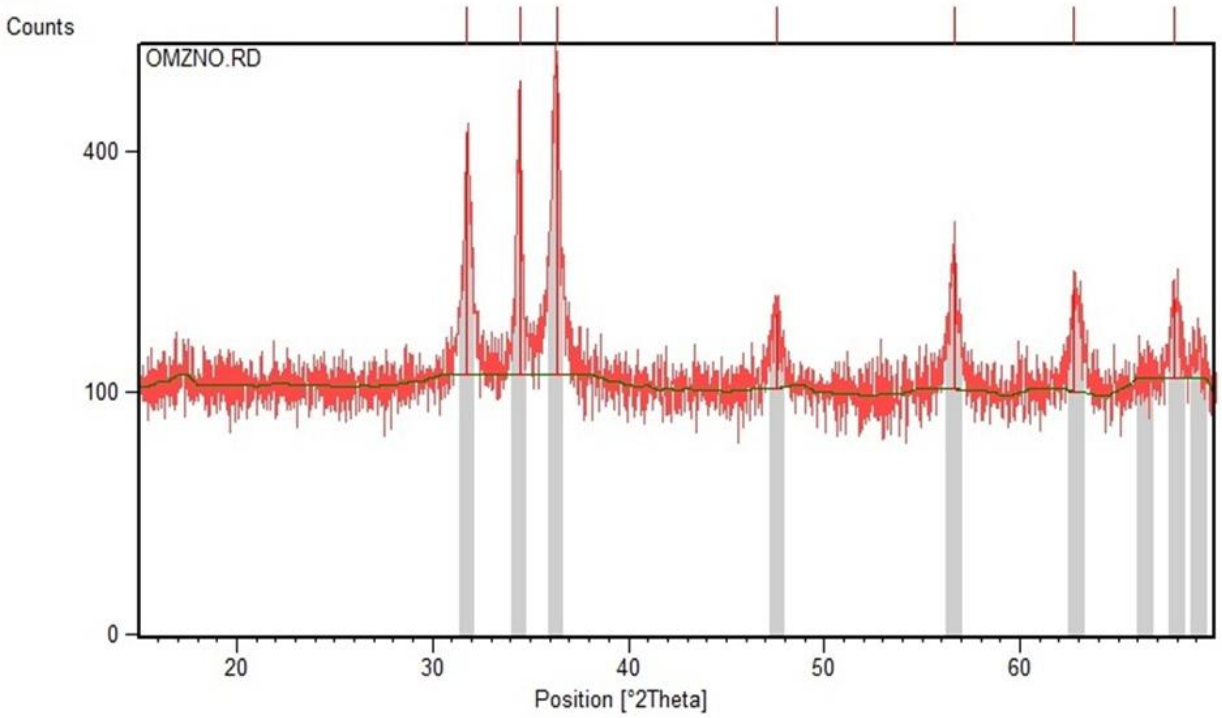

Figure 2: XRD analysis graph

By applying Scherers formula, the sizeof the crystallite of the ZnO samples were found to be same as the value measured by particle size analyzer. Here it

was seen that the diffractograms were almost same but there were some undesired picksin between, which was most probably because of the impurities present in the samples.

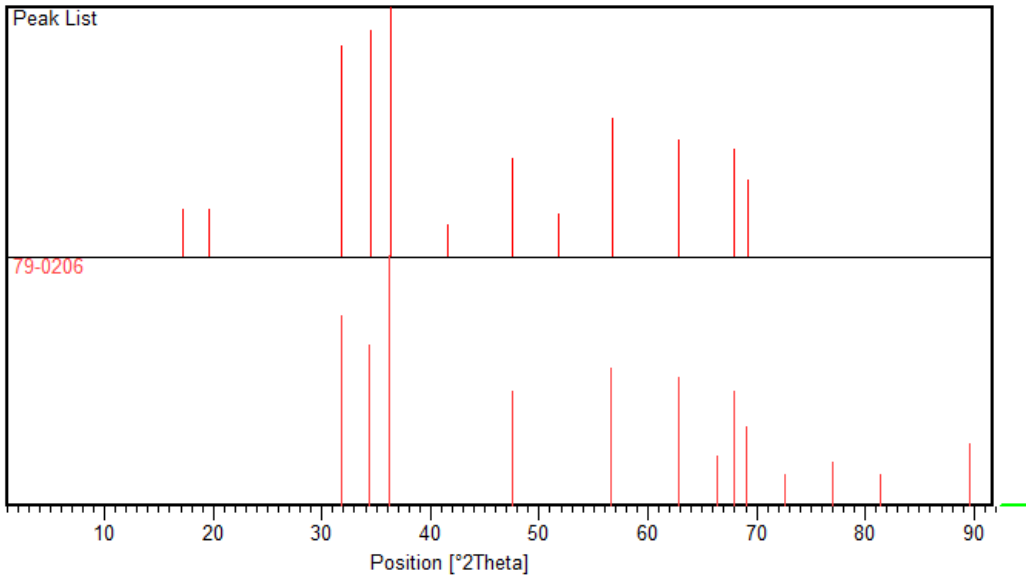

Figure 3: XRD analysis

Pattern List | Scan List | Peak List | Anchor Scan Data

No.	Pos. [°2Th.]	Height [cts]	FWHM [°2..	d-spacing [Å]	Rel. Int. [%]	Tip width [°..	Matched by
1	31.7760	315.74	0.1200	2.81379	71.29	0.1000	79-0206
2	34.4664	412.08	0.1200	2.60007	93.04	0.1000	79-0206
3	36.3198	442.93	0.3840	2.47152	100.00	0.3200	79-0206
4	47.5607	72.90	0.2880	1.91031	16.46	0.2400	79-0206
5	56.6622	145.80	0.2880	1.62316	32.92	0.2400	79-0206
6	62.7680	103.04	0.5760	1.47915	23.26	0.4800	79-0206
7	67.8922	79.57	0.5760	1.37944	17.97	0.4800	79-0206

Figure 4: XRD analysis table

7.2 Scanning electron microscope (SEM)

The scanning electron microscope uses a beam of high-energy electrons to produce a variety of signals at the surface of specimens used. The signals show information about the sample including chemical composition, and crystalline structure, external morphology(texture) and orientation of materials which make up the sample. SEM analysis is normally considered to be non-destructive because the x-rays generated do not lead to loss of volume of the sample, so it becomes possible to repeatedly analyze the same materials. A scanning electron microscope is a kind of electron microscope which images a sample by scanning it using a high-energy electron beam. The electrons then interact with the atoms making up the sample, thus producing signals which reveal information about the sample's composition, surface topography and other properties such as electrical conductivity.

Various types of signals produced by a SEM include back-scattered electrons (BSE), secondary electrons, characteristic X-rays, specimen current, light (cathodoluminescence) and transmitted electrons. Back-scattered electrons (BSE) are the electrons which are reflected by elastic scattering from the sample. Because the intensity of the BSE signal is related to the atomic number of the specimen, BSE images can provide information about the different elements distribution in the sample very accurately. Characteristic X-rays are released when the electron beam removes an electron from the inner shell of the sample, thus causing a higher energy electron to occupy the shell and hence release energy in the form of Xrays. These characteristic X-rays are in turn used to find out the composition of the material and also measure the presence of elements in the sample as well as the level of impurities.

Magnification in a scanning electron microscope technique can be controlled over a range of about 6 orders of magnitude from approximately 10 to 500,000 times. Assuming that the display screen has a fixed size, higher magnification is obtained by reducing the raster size of the specimen, and vice versa. Magnification is hence controlled by the voltage supplied to the x, y deflector plates or the current supplied to the scanning coils and not by objective lens power.

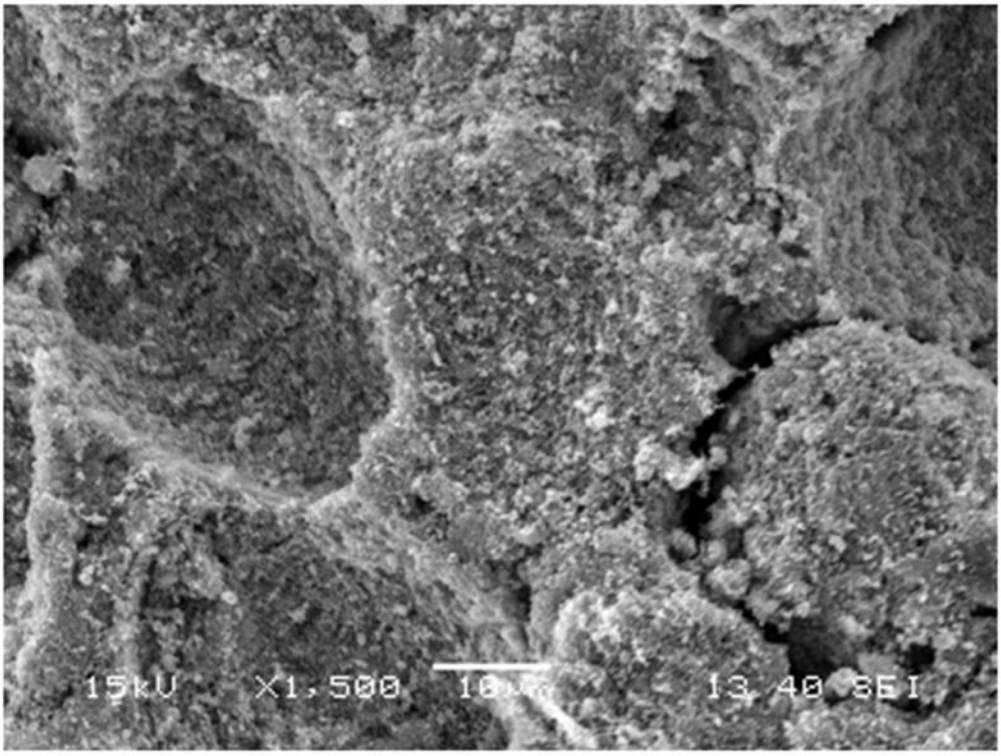

Figure 5: SEM analysis

Conclusion

8 Conclusion

Zinc Oxide was synthesized by precipitation method from Zinc Nitrate and soluble starch using Sodium Hydroxide.A white colour ZnO powder was obtained .Characterization study was carried out using XRD and SEM. The XRD patterns are used for phase identification and they showed amount of impurities and structure depending on the peaks present in the structure.ZnO was found to be hexagonal in structure. XRD Scherer's formula is used to find particle size of ZnO($0.9\lambda/(B^*\cos\theta)$). Size was found to be 21.7 nanometer.Crystaline peaks showed ZnO nanoparticles and rest are amorphous.SEM was used for microstructure studyand to find agglomeration. Nanoparticles showed small amount of agglomeration. This paper intends to develop an innovative and more appropriate nanoparticles synthetic procedure and characterisation under favourable conditions that helps in biological systems

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